Effect of Live Yeast *Saccharomyces cerevisiae* on Post-weaning Performance, Diarrhea and Immune Parameters with an Environmental Challenge

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Abstract

Weaning imposes multiple stressors that reduce feed intake and impair intestinal integrity. Furthermore, poor environmental management could compound the high stress period increasing morbidity and mortality of postweaning piglets. The objective of this research was to investigate the effect of supplemental *Saccharomyces cerevisiae* on postweaning growth performance, fecal scores and immune parameters in a clean or dirty nursery environment. The experiment was a 2 X 2 factorial design with two dietary treatments fed in a sanitized (following barn SOP) and un-sanitized (pits flushed, feeders and pens scraped) nursery environment. Weaned piglets (n=260 and 5pigs/pen; 20.8 day of age) were allotted to the following dietary treatments: 1) control or 2) *Saccharomyces cerevisiae* (0.1% in phase 1 and 2 and 0.05% phase 3 diets) for 5-wks postweaning. On days 3, 7, 14, 21 and 35 fecal scores/pen and blood samples were collected for monitoring diarrhea and measurement of cytokines. Overall, pigs fed *Saccharomyces cerevisiae* had greater ADG compared to control fed pigs regardless of environment (P= 0.09; 379 vs. 357 g/d, *Saccharomyces cerevisiae* vs control, respectively), and final pen weights at d35 were greater in *Saccharomyces cerevisiae* vs. control fed pigs (101 vs. 97 kg/pen; $P < 0.05$). Pigs reared in the dirty environment vs the clean environment had reduced overall ADG (352 vs 384 g/d, respectively; $P = 0.01$), and pigs raised in the dirty environment had an overall greater F:G ratio compared to pigs raised in clean environments; 1.87 vs. 1.76 g/g ($P = 0.09$). Diarrhea scores were increased in the dirty environment compared to the clean environment on days 3 and 7 ($P < 0.01$). Serum tumor necrosis factor alpha (TNF-α) concentrations were not significantly affected by diet or environment. In conclusion, *Saccharomyces cerevisiae* increased overall ADG in weaned pigs regardless of environment and environmental challenge reduced growth and efficiency parameters in the nursery pigs.

Introduction

Piglet weaning stress is one of the most challenging times in swine production where multiple stressors impact gut health and creates an environment for opportunistic pathogens to exacerbate intestinal inflammation leading to further disruption of intestinal barrier function[1]. Enterotoxigenic Escherichia coli (ETEC) infections are a major cause of post-weaning diarrhea (PWD) in nursery piglets worldwide[2]. Upon attachment of ETEC to the intestinal epithelium, subsequent pathogen proliferation and secretion of enterotoxins occurs and results in secretory diarrhea in nursery pigs[3]. Tight junctions constitute the intestinal barrier and control the types of solutes and molecules which move across the intestinal epithelial cells. When the intestinal barrier is damaged, the performance of tight junctions and barrier are compromised. As a result, intestinal cells are unable to effectively digest, absorb, and utilize nutrients. Not only does poor intestinal function stunt the growth and development of the animal, it can lead to diarrheal diseases, which are very devastating to the swine industry[4].

Antibiotic use has been shown to decrease the incidence of diarrheal disease in a nursery setting. However, there is a pressure to remove antibiotics as growth promoters in the livestock industry due to consumer pressure and concern for antibiotic resistance. This has major implications for weaned pigs compared to other production stages due to social and environmental stressors which affect gut barrier function and the immune system adversely, and lead to increased incidence of disease, including diarrhea[5]. The control and prevention of ETEC infections using antibiotics has been used for many years; however, alternative methods are necessary.

Previous research has demonstrated the preventative and therapeutic effects of live yeast probiotics such as *Saccharomyces spp*[6]. When supplemented in a nursery environment,

Saccharomyces spp. decreased diarrhea scores, duration of diarrhea, and pathogenic *Escherichia coli* (O149:K88) shedding[7]. Furthermore, yeast probiotics exhibited anti-inflammatory properties in porcine intestinal cell lines (IPEC-1) after challenge with enterotoxigenic E. coli (K88+) by increasing anti-inflammatory mediators[8]. Supplemental dietary probiotics show potential to serve as an alternative for antibiotics currently used in the swine industry.

Problem Identification and Justification

Post-weaning diarrhea is the most prevalent disease of piglets in the nursery and contribute a major economic burden to the swine industry[9]. Dietary supplementation with antibiotics serves as an effective strategy for preventing PWD, but with increasing public concern for antibiotic resistance, this form of supplementation is limited. Finding acceptable alternatives to antibiotics is important to the livestock industry and dietary probiotics demonstrate promise as a safe and feasible replacement.

Objectives and Hypothesis

The objective of this research project was to determine if the daily administration of dietary live yeast, *Saccharomyces cerevisiae*, would provide an effective alternative to antibiotics currently used to treat diarrheal diseases as well as improve weight gain and growth performance in different farm management systems. We hypothesized that the yeast probiotic-fed pigs would have a better growth performance and improved feed conversion ratio than pigs fed the control diet. Furthermore, pigs were housed in one of two nursery environments; 1) control, a thoroughly cleaned and disinfected room, or 2) challenged environment, a room that has not been cleaned following the previous set of pigs. We hypothesized that the pigs reared in the challenged

environment will have less diarrhea and improved growth rate when offered the yeast diet compared to control diet-fed pigs.

Methods

Two hundred and sixty weaned piglets (~18-21 d old, initial BW (body weight) = 6.68 ± 0.67 kg) were weighed at weaning and sorted such that treatment was balanced for litter origin, BW, and sex. Piglets were allotted to one of four treatments in a 2 x 2 factorial arrangement and included 1) control diet, clean environment 2) ActiSaf HR+ diet, clean environment 3) control diet, dirty environment 4) ActiSafHR+ diet, dirty environment. Piglets were housed in environmentally controlled nursey rooms with ad libitum access to feed and water at all times. Pens had plastic slatted floors and were equipped with a self-feeder and two nipple waterers. The dirty nursery was not cleaned after the previous nursery group was removed. The clean nursery was cleaned following standard cleaning protocol at the Don Scott Swine Research Center. This animal use protocol was approved by The Institutional Animal Care and Use Committee (IACUC) at Ohio State University.

Piglets were fed diets formulated to meet the NRC requirements for nursery pigs and were fed in three phases (Table 1; NRC, 2012). Phases 1, 2, 3 took place during d 1 to 7, d 7 to 21, and d 21 to 35, respectively. Yeast supplementation was added to diets at 0.1% (1×10^{10} CFU/kg diet) in phase 1 and 2, and 0.05% (5×10^{9} CFU/kg diet) in phase 3. All diets were pelleted and crumbled at the Ohio State University feed mill located in Wooster, OH. Pig BW and feed disappearance data were collected at the end of weeks one, two, three, and five.

Feed and performance data and pen fecal scores were collected on days zero, seven, fourteen, twenty-one, and thirty-five. Fecal scores were based on a scoring system (0, dry, hard, well-formed feces; 1, soft, but formed feces; 2, pasty feces green or brown in color; 3, viscous feces light in color, episodic; 4, fluid feces in light color; 5, watery feces, continuous). Piglet health, mortality, and morbidity was monitored and recorded throughout the trial. Only upon advisement of the staff veterinarian were antibiotic treatments administered and all treatments were recorded.

On days three, seven, fourteen, twenty-one, and thirty-five, blood from two piglets, one male and one female, in each pen was collected via jugular vein puncture. Blood was centrifuged, and the serum was pipetted into 2-mL tubes and stored at -80°C for future analysis. Concentration of TNF-α within the serum was analyzed via Invitrogen Porcine ELISA kits (Fisher Scientific, Hampton, NH).

Individual pens served as the experimental unit and thirteen pens within each of the four treatments served as BW and sex block. Altogether, there was a total of 52 pens across all four treatments. Growth data, weight and TNF-α measures were analyzed as a 2 x 2 factorial arrangement in a randomized block design with repeated measures in time using the PROC MIXED procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). The model included fixed effects of diet, environment, and phase of diet, and their interactions. Random effects included pen nested within diet, environment and block. Various covariate structures of error were fitted, and compound symmetry was selected based on the lowest Bayesian Information Criterion (BIC). P-values of less than 0.05 were considered statistically significant for this study.

Results

Growth Performance

A trend for an environment by phase interaction (P = 0.07; Table 2) was observed. Furthermore, there is a significant environmental effect for ADFI (average daily feed intake) and ADG (average daily gain) (P < 0.0001; Table 2). A trend for F:G (feed to gain ratio) in the phase analysis is observed (P = 0.8; Table 2). Pigs fed ActiSaf HR+ had greater ADG compared to control fed pigs regardless of environment (P= 0.09; Table 2; 379 vs. 357 g/d, ActiSaf vs control, respectively). Furthermore, final pen weights at d35 were greater in ActiSaf vs. control fed pigs (101 vs. 97 kg/pen; P < 0.05; Table 2). In regard to environmental challenge, pigs reared in the dirty environment versus the clean environment had reduced overall ADG (352 vs 384 g/d, respectively; P = 0.01). Similarly, pigs raised in the dirty environment had an overall greater F:G (feed to gain) ratio compared to pigs raised in clean environments 1.87 vs. 1.76 g/g (P = 0.09; Table 3).

Fecal Scores and TNF-α Concentrations

Diarrhea scores were increased in the dirty environment compared to the clean environment on days 3 and 7 (P < 0.01; Figure 2)). However, serum tumor necrosis factor alpha (TNF-α) concentrations were not significantly affected by diet or environment (Figure 3).

Discussion

Because feed cost is the largest expense in pork production, it is important to consider what health benefits a dietary supplement may confer on an animal as well as how effectively it improves growth parameters. Feed efficiency drives profitability and each point of improvement in whole herd feed conversion (2.90 to 2.89) represents ~1.6kg (3.52 lb) of feed per pig or 186 million tons per year for the whole industry. The cost saving amounts to $0.40/pig or $47 million[10].

While this project only observed the nursery portion of the production life cycle of a pig, improved gut health early in life that improves feed efficiency could also drive improved feed efficiency across the life span of the pig. Furthermore, when pigs leave the nursery at higher BW, they get to finish at a faster rate, thus shortening the feeding window a producer needs to feed.

ActiSaf HR+ increased overall ADG in weaned pigs regardless of environment, and environmental challenge reduced growth and efficiency parameters in the nursery pigs. Both trends and significant environmental effects were observed for ADFI, ADG, and F:G in the phase analysis, which shows how proper management of animals is crucial. Poor management can have significant negative impact on performance not only acutely, but also over the entire growth trial. Increased diarrhea scores in the dirty environment as compared to the clean environment additionally establishes the importance of proper management. Nursery management is critical to good performance of pigs[11]. A clean environment can decrease additional stress and disease factors on pigs at weaning. It is significant to ensure additional stress, disease factors on the pig transitions at weaning are minimized, and this can be done by having a clean environment.

In conclusion, yeast probiotics demonstrate beneficial effects on growth performance as Actisaf HR+ diet improved performance, even in clean environment. As hypothesized, yeast probiotic-fed pigs had better growth performance parameters and improved feed conversion ratio than pigs fed the control diet. This is potentially due to the ability of the yeast probiotic to improve gut health by conferring anti-inflammatory properties to the host.

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Table 1. Calculated Diet Table

	Phase 1	Phase 2	Phase 3
Weight of Pigs	12 -15 lb	15-25 lb	25-50 lb
Ingredient			
Corn	718	1001	1206
Soybean Meal, Dehull, Sol Extr	380	535	685
Bovine Blood Plasma	80		
Corn DDGS, >6 and <9% Oil	100		
Fish Meal Combined	50		
Milk, Whey Powder	500	200	
Choice White Grease	60	40	40
Calcium phosphate (monocalcium)	15	21	23
Limestone, ground	15.5	22	20
Sodium chloride	6	6	7
L-Lys-HCL	4.5	6	6
DL-Met	2.8	3.2	2.5
L-Thr	1.5	2.2	2.3
Trace mineral premix	3	3	3
Vitamin premix without phytase	5	5	5
Choline chloride 60%	0.7		
HiPhos 2700		0.3	0.3
Zinc oxide	7.8	5	
HP 300 (Hamlet Protein)	50	150	
TOTAL	2000.0	2000.0	2000.0
Required SID Lys:NE Ratio	5.65	5.48	5.04
Calculated SID Lysine Required, %	1.47	1.37	1.25

Table 2. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on pig performance.

Environment	Clean		Dirty			P values						
Diet	Control	ActiSaf	Control	ActiSaf	SEM	Diet	Environ.	Phase	Diet*Environ	Diet*Phase	Environ* Phase	Diet*Environ*Phase
ADFI, g						0.123	0.081	< 0.0001	0.531	0.161	0.072	0.510
d 1 to 7	257.67	253.96	232.39	236.12	33.3							
d 7 to 21	541.99	587.51	539.10	567.91	33.3							
d 21 to 35	978.47	1095.80	923.51	958.88	33.3							
d 1 to 35	659.18	697.05	629.11	663.31	24.6	0.154	0.206		0.941			
ADG, g						0.424	0.035	<0.0001	0.190	0.489	0.528	0.809
d 1 to 7	220.35	81.99	6.34	26.15	58.7							
d 7 to 21	436.59	332.15	322.68	314.97	58.7							
d 21 to 35	624.96	611.42	531.07	582.81	58.7							
d 1 to 35	375.56	391.81	337.70	365.13	12.6	0.089	0.013		0.658			
F:G, g/g						0.387	0.067	0.798	0.230	0.406	0.051	0.168
d 1 to 7	1.95	11.11	-0.08	-1.62	2.3							
d 7 to 21	2.47	1.88	1.75	1.97	2.3							
d 21 to 35	1.76	1.83	1.79	1.68	2.3							
d 1 to 35	1.75	1.77	1.90	1.84	0.06	0.732	0.094		0.502			

Table 3. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on pen weight (kg).

Environment	Clean		Dirty			P values						
Diet	Control	ActiSaf	Control	ActiSaf	SEM	Diet	Environ.	Phase	Diet*Environ	Diet*Phase	Environ* Phase	Diet*Environ*Phase
Pen Weight, kg						0.668	0.406	<.0001	0.692	0.018	0.012	0.358
Day 1	33.33	33.39	33.45	33.43	2.26							
Day 7	41.04	36.25	34.02	34.26	2.26							
Day 14	43.37	44.09	42.33	46.35	2.26							
Day 21	58.11	59.35	57.3	57.23	2.26							
Day 35	98.93	102.01	95.35	99.46	2.26							

Figure 2. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on fecal scores. The severity of diarrhea in each pen was scored on a scale of one to five: zero, dry, hard, well-formed feces; one, soft but formed feces; two, pasty feces green or brown in color; three, viscous feces in light color, episodic; four, fluid feces in light color; five, watery feces. This score will be an average of the pen. Bars represent means ± SEM.

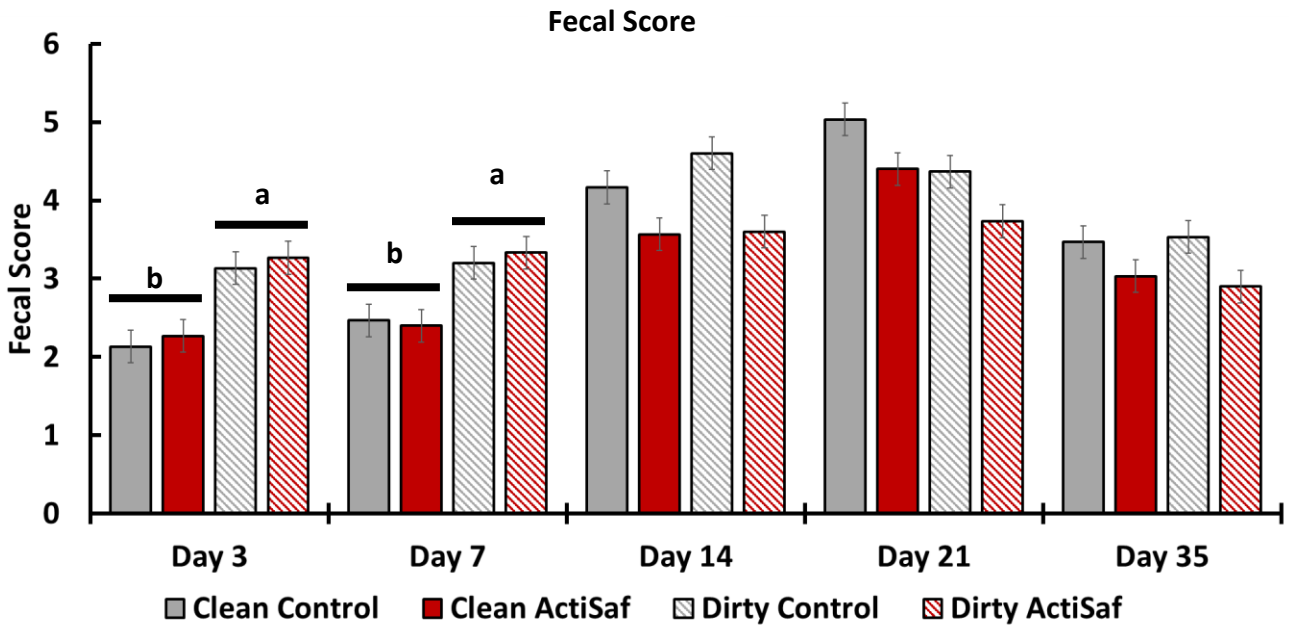

Figure 3. Effect of dietary supplementation of ActiSaf HR+ in a clean compared to a dirty nursery environment on tumor necrosis factor-α. Bars represent means ± SEM.